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A powerful and rapid approach to human genome scanning using small quantities of genomic DNA

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Summary

Dense maps of short-tandem-repeat polymorphisms (STRPs) have allowed genome-wide searches for genes involved in a great variety of diseases with genetic influences, including common complex diseases. Generally for this purpose, marker sets with a 10 cM spacing are genotyped in hundreds of individuals. We have performed power simulations to estimate the maximum possible inter-marker distance that still allows for sufficient power. In this paper we further report on modifications of previously published protocols, resulting in a powerful screening set containing 229 STRPs with an average spacing of 18·3 cM. A complete genome scan using our protocol requires only 80 multiplex PCR reactions which are all carried out using one set of conditions and which do not contain overlapping marker allele sizes. The multiplex PCR reactions are grouped by sets of chromosomes, which enables on-line statistical analysis of a set of chromosomes, as sets of chromosomes are being genotyped. A genome scan following this modified protocol can be performed using a maximum amount of 2·5 μg of genomic DNA per individual, isolated from either blood or from mouth swabs.

1. Introduction

The localization of genes in genome-wide searches is performed by the use of saturated maps of short-tandem-repeat polymorphisms (STRPs) or microsatellite loci. Recent applications of STRP maps are genome scans for genes involved in common multifactorial diseases such as type II diabetes (Mahtani *et al.*, 1996; Ghosh *et al.*, 1998, 1999) and osteoarthritis (Chapman *et al.*, 1999) or for quantitative trait loci (QTL) determining quantitative variables associated with disease risk (Xu *et al.*, 1999).

Genome scans thus far comprise genotypings of on average 400 microsatellite marker loci at 10 cM spacing in hundreds of individuals and are extremely time-consuming. To increase the efficiency, a balanced choice of markers can be made based on the maximum possible spacing to limit the number of genotypings at a minimum risk of type II errors. To minimize genotyping errors, tri- and tetranucleotide repeat polymorphisms can more accurately be scored than CA-repeat polymorphisms, because of greatly reduced strand slippage during amplification and concomitant simplification of banding patterns. Additionally, CA-repeat loci have a mutation rate 1·5–2 times higher than tetranucleotide repeat loci (Chakraborty *et al.*, 1997). Multiplex PCR reactions, amplifying several STRPs simultaneously, should be developed in which markers in the same reaction do not overlap.

We have modified existing protocols of Human Screening Set 8 (Yuan *et al.*, 1997), developed by the Marshfield Medical Research Foundation, for genome scanning in which minimal amounts of genomic DNA, including DNA collected by the non-invasive mouth swab procedure (Meulenbelt *et al.*, 1995), are required.

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2. Materials and methods

(i) *Power simulation*

Power simulations were based on 500 unselected sib pairs and a trait heritability of 75%. Simulations were carried out for markers with a heterozygosity of 75%. The QTL effect accounted for 25% of the phenotypic variance and the QTL was always located between two loci. Data were analyzed using a maximum-likelihood variance components approach modelling the full 2×2 sibship covariance structure (Fulker & Cherny, 1996), with pi-hats estimated from IBD distributions obtained from running Mapmaker/SIBS (Kruglyak & Lander, 1995).

(ii) *Marker set*

The Human Screening Set 8 (Yuan *et al*., 1997) was used as a basis for composing the alternative screening set. The primer sequences and the inter-marker distances are available at the Marshfield Medical Research Foundation web site (http://research.marshfieldclinic.org/genetics).

(iii) *DNA isolation*

Genomic DNA was isolated from whole blood and from mouth swabs using a chloroform:isoamylalcohol (24:1) extraction method (see below). Mouth swabs were taken from subjects according to our previously published method (Meulenbelt *et al*., 1995), but since the chloroform:isoamylalcohol (24:1) extraction method appeared to be as successful as the phenol/chloroform extraction we used the former. The mouth swabs were collected from participants by post. Mouth swabs were taken and stored by the participants in STE buffer (100 mM NaCl, 10 mM EDTA, 10 mM Tris) including 0·1 mg/ml proteinase K and 0·5% SDS. When the swabs arrived by post in the TNO laboratories, the proteinase K concentration was increased to 0·2 mg/ml and the swabs were incubated at 65 °C for 2 h. After centrifugation of the samples as described (Meulenbelt *et al*., 1995), 0·2 volume of 8 M KAc was added to the lysate and mixed well but gently. The mixture was kept on ice for 15 min. One volume of chloroform:isoamylalcohol (24:1) was added and placed in a top-over for half an hour. Subsequently, samples were centrifuged and isopropanol was added to the supernatant for precipitation of genomic DNA. DNA of 1911 individuals was obtained using this mouth swab procedure and the average yield per cotton bud was 2·0 μg (SD 1·4) genomic DNA. Depending on the subject taking the mouth swab, the DNA yield is quite variable. For DNA extraction from whole blood lysis was performed as usual, followed by the same extraction steps as for mouth swabs.

(iv) *PCR conditions*

The PCR was performed in 96 wells V-microtitre plates (Biozyme) in a total volume of 10 μl. The reaction mix contained 10 ng genomic DNA template, isolated from either whole blood or mouth swabs; PCR buffer as supplied with the enzyme by the manufacturer (Amersham Pharmacia Biotech); 0·073 μM of each primer, of which the forward primer was labelled with Cy5 (Amersham Pharmacia Biotech); 200 μM each of dATP, dTTP, dGTP, dCTP (Amersham Pharmacia Biotech); and 0·2 units recombinant *Taq* DNA polymerase (Amersham Pharmacia Biotech). To make the multiplex PCR reaction successful, it was only necessary in some cases to adapt the final primer concentration. The PCR reactions were performed in PTC-100 machines (MJ Research). The program consisted of an initial denaturation for 1 min at 94 °C, followed by 27 cycles of 30 s denaturing at 94 °C, 75 s annealing at 55 °C and 15 s extension at 72 °C, which were concluded with a final extension of 6 min at 72 °C.

(v) *Electrophoresis*

The electrophoresis and fragment separation were performed using short gel systems of the automated laser fluorescent DNA sequence analyzer ALF*express* (Amersham Pharmacia Biotech). When Sequagel-6 (National Diagnostics) was used, the gel could be loaded twice with PCR products and when High Resolution ReproGel (Amersham Pharmacia Biotech, which polymerizes during 10 min of exposure to UV light, was used, the gel could be loaded at least three times in subsequent order, still resulting in sharp peak patterns.

(vi) *Genotyping*

The allele analysis was performed using Fragment Analyser software (Amersham Pharmacia Biotech). The genotypes of the parents of eight CEPH families (102, 884, 1331, 1332, 1347, 1362, 1413 and 1416) were used for composing the allelic standards. Each genotype was reviewed manually by two individuals to confirm the accuracy of allele calling.

3. Results

Simulations showed that genome scans with a spacing of 20 cM have enough power to detect QTL effects, which account for 25% of the phenotypic variance of

Table 1. *Composition of the screening sets of chromosomes*

Set of chromosomes	Multiplex reaction numbers
1, 8, 11, 19	1–23, 42, 43, 67
6, 7, 16	6, 9, 10, 13, 14, 18, 20, 24–31
2, 15, 17	7, 8, 10, 13, 18, 32–43
5, 9, 14	12, 16, 19, 40, 41, 44–54, 78
3, 4, 10	11, 15, 29, 40, 51, 55–64, 78
12, 13, 18	6, 12, 52–54, 62, 65–72
20, 21, 22, X, Y	15, 17, 21, 52, 54, 62, 72–80

a trait with a heritability of 75%, in a population of 500 sib pairs. The 25 cM Human Screening Set 8a appears to have too little power in searches for such QTLs and the 10 cM Human Screening Set 8 (Yuan *et al*., 1997) seems too elaborate. Therefore, we composed an alternative set of 229 markers based on the Human Screening Set 8 with an average spacing of 18·3 cM (SD 3·9). The average heterozygosity of these markers is 0·77 (SD 0·06) and 86·5% are tri- and tetranucleotide repeat polymorphisms.

The Marshfield Medical Research Foundation described multiplex PCR combinations for their 10 cM spaced screening set (http://research.marshfieldclinic.org/genetics). We redesigned the composition of markers in each multiplex PCR and the corresponding conditions. The resulting 80 multiplex PCR reactions (Appendix) are grouped by markers randomized over sets of 3 to 5 chromosomes (Table 1) to enable statistical analysis of the search data before finishing the whole genome scan at large. In addition, a margin of at least 15 base pairs between allele sizes of different markers in a multiplex was chosen to avoid overlap.

A protocol was developed in which only 10 ng genomic DNA per multiplex PCR reaction is used. Hence, a complete genome scan requires at most 2·5 μg of genomic DNA. Our protocol is successful using DNA isolated from whole blood as well as from mouth swabs. After evaluation of 90000 genotypes, which were performed in 16 months using two ALF*express* (Amersham Pharmacia Biotech) systems, the average genotypic error rate was less than 1% and the average missing data rate was 8% using this protocol.

4. Discussion

We performed power simulations and adapted existing protocols in order to limit the number of genotypings in a genome scan and still retain sufficient power in statistical analysis. This resulted in a set of 229 markers, measured in 80 multiplex PCR reactions, with an average inter-marker distance of 18·3 cM. The high percentage of tri- and tetranucleotide repeat markers reduces the percentage of genotypic errors. The markers in each multiplex PCR reaction, which all require the same PCR conditions, allow 15 base pairs spacing of alleles to avoid overlap. The genotypings were performed using an ALF*express* system (Amersham Pharmacia Biotech), but these multiplex PCR reactions can easily be used in other genotyping equipment.

Multiplex PCR reactions were composed from marker combinations grouped by sets of several chromosomes. Statistical analysis can therefore be performed per set of chromosomes, while other sets are being genotyped. Using our protocol, fine-mapping of positive chromosome regions could be started at an earlier stage.

Due to the adapted PCR conditions, smaller than usual amounts of DNA are required. For a whole-genome scan using our protocol, at most 2·5 μg of genomic DNA is required, whereas for a 10 cM spaced genome scan of the Marshfield Medical Research Foundation (http://research.marshfieldclinic.org/genetics), a 6-fold increment of genomic DNA is required.

Finally, our genotyping procedure is successful on genomic DNA isolated from blood as well as from mouth swabs collected by post. The use of DNA isolated from mouth swabs is especially suitable for studies involved in geographically scattered subjects and for studies in which it is not feasible to obtain blood from participants. In conclusion, we have increased the efficiency of genome scanning and developed a protocol to facilitate scanning on small quantities of genomic DNA.

Appendix. *List of loci in multiplex reactions*

Reaction number	Chromosome	Marker name	Locus name
1	8	GAAT1A4	
	11	GATA28D01	D11S2000
	1	GATA72H07	D1S2134
2	8	GATA26E03	D8S1132
	11	GATA90D07	D11S2371
	1	GATA87F04	D1S2141
3	8	143XD8	D8S264
	19	GATA29B01	D19S589
	11	GATA6B09	D11S1392
	1	GATA4A09	D1S547
4	1	ATA29D04	D1S1631
	8	GATA14E09	D8S2324
5	1	GGAA3A07	D1S1612
	8	UT721	D8S373
	11	GATA26H10	D2S2739
6	8	COS140D4	D8S136
	11	GATA23F06	D19S1999
	18	ACT1A01	D18S843
	6	ATA50C05	D6S2434
7	8	GATA25C10	D8S1130

	1	GATA50F11	D1S1609		16	GATA67G11	D16S2620
	17	AFM044XG3	D17S784	32	15	ACTC	ACTC
8	8	GGAA20C10	D8S1477		17	ATA43A10	D17S2193
	11	ATA27C09	D11S2359		2	GATA116B01	D2S2952
	1	ATA29C07	D1S3462	33	2	AFM112YD4	D2S125
	2	GATA71D01	D2S1776		17	AFM217YD10	D17S928
9	6	GATA31	D6S474		15	GATA27A03	D15S642
	1	GGAA22G10	D1S1677	34	2	GATA30E06	D2S2944
	19	GATA66B04	D19S714		15	GATA63A03	D15S659
10	17	GATA185H04	D17S2196		17	GATA64B04	D17S1303
	7	GATA189C06	D7S3070	35	17	ATC6A06	D17S2180
	1	GATA48B01	D1S1660		2	GATA86E02	D2S1788
11	10	ATA31G11	D10S1412		15	GATA88H02	D15S822
	1	GATA12A07	D1S534	36	2	GATA11H10	D2S1360
	4	GATA28F03	D4S3248		15	AFM323YD9	D15S211
	3	GGAT2G03	D3S2406		17	GTAT1A05	D17S1308
12	13	GATA51B02	D13S796	37	17	GATA28D11	D17S1301
	1	ATA4E02	D1S1589		2	GATA165C07	D2S2976
	5	AFM164X68	D5S408		15	ATA24A08	D15S652
13	2	GGAA20G04	D2S1399	38	17	GATA49C09	D17S1290
	8	AFM073Y67	D8S256		2	GATA4D07	D2S1334
	1	ATA20F08	D1S1622		15	GATA22F01	D15S657
	6	GATA64D02	D6S1053	39	2	GATA4E11	D2S410
14	6	GGAT3H10	D6S1017		15	GATA151F03	D15S1507
	7	MFD265	D7S559		17	GAAT2C03	D17S1298
	1	GATA165C03	D1S3728	40	10	GGAA5D10	D10S1213
15	X	GATA31F01	DXS6789		2	GATA69E12	D2S1394
	1	GATA29A05	D1S3669		3	GATA22F11	D3S2427
	4	ATA26B08	D4S2394		9	GATA62F03	D9S2169
16	1	AFM280WE5	D1S468	41	2	GATA178G09	D2S2968
	5	GATA145D09	D5S2848		5	ATA20G07	D5S2488
17	19	GATA44F10	D19S591	42	19	MFD238	D19S254
	1	GATA6A05	D1S551		2	GATA23D03	D2S1363
	X	GATA69C12	DXS6810	43	19	MFD232	D19S246
18	2	GATA65C03	D2S1391		2	GATA88G05	D2S1790
	16	AFM031XA5	D16S402	44	5	GATA21D04	D5S1457
	8	GGAA8G07	D8S1113		9	GATA27A11	D9S925
19	8	GATA7G07	D8S1179		14	ATA19H08	D14S592
	9	AFM308VB1	D9S282	45	5	GATA52A12	D5S1501
20	11	AFM157XH6	D11S912		14	GATA136B01	D14S1426
	16	GATA22F09	D16S3253		9	GATA87E02	D9S1121
	6	GATA68H04	D6S1056	46	9	ATA18A07	D9S910
	7	GATA118G10	D7S3046		14	GATA4B04	D14S306
21	11	GATA23E06	D11S1998	47	5	GATA67D03	D5S2500
	X	AFM150F10	DXS1047		9	AFM73YB11	D9S158
22	11	GGAA17G05	D11S1984		14	GATA74E02	D14S742
	19	GATA21G05	D19S1034	48	14	MFD190	D14S53
23	11	GATA48E02	D11S1981		5	MFD154	D5S211
24	16	AFM350VD1	D16S516		9	GATA21F05	D9S922
	7	GATA3F01	D7S820	49	14	GGAA21G11	D14S617
	6	GATA81B01	D6S1277		9	GATA7D12	D9S301
25	6	ATA22G07	D6S1027		5	GATA3E10	D5S817
	7	GATA24F03	D7S3056	50	14	GATA43H01	D14S608
	16	GATA71F09	D16S2621		9	ATA59H06	D9S2157
26	6	GATA165G02	D6S2436		5	GATA68A03	D5S2501
	7	GATA41G07	D7S1802	51	3	GATA27C08	D3S2432
	16	GATA71H05	D16S769		5	GATA6E05	D5S820
27	16	ATA41E04	D16S2616		9	GATA48D07	D9S930
	7	GATA31A10	D7S2846	52	20	GATA81E09	D20S604
28	16	GATA42E11	D16S764		5	GATA3A04	D5S807
	7	GATA32C12	D7S1824		12	PAH	PAH
	6	GATA32B03	D6S1009	53	12	GATA26D02	D12S1052
29	3	GATA22G12	D3S2387		5	GATA89G08	D5S1725
	7	GATA5D08	D7S821		13	GATA43H03	D13S793
30	7	AFM217YC5	D7S513	54	20	GATA51D03	D20S482
	6	GATA163B10	D6S2439		5	GATA11A11	D5S1456
31	7	GGAA6D03	D7S3061		18	GATA6D09	D18S851
	6	F13A1	SE30	55	3	AFM254VE1	D3S1311

	4	GATA107	D4S1625
	10	GATA115E01	D10S2470
56	4	GATA22G05	D4S2366
	3	GATA68D03	D3S2459
	10	GGAA23C05	D10S1248
57	4	GATA72G09	D4S2632
	3	ATA34G06	D3S4523
	10	GATA48G07	D10S1237
58	4	GATA90B10	D4S2639
	10	GATA70E11	D10S1423
	3	GATA3H01	D3S1763
59	4	GATA10G07	D4S3243
	3	GATA6F06	D3S1766
	10	GATA87G01	D10S1435
60	4	GATA2F11	D4S1647
	10	ATA24F10	D10S1225
	3	GATA4A10	D3S1764
61	10	ATA5A04	D10S1208
	4	AFM165XC11	D4S408
	3	GATA6G12	D3S2398
62	4	GATA5B02	D4S1652
	X	GATA175D03	DXS9902
	12	GATA11H08	D12S391
63	4	GATA8A05	D4S1629
	10	AFM189ZB4	D10S212
	3	AFM234TF4	D3S1304
64	X	GATA124E07	DXS9896
	4	GGAA19H07	D4S2431
65	12	ATA25F09	D12S2070
	18	ATA7D07	D18S1357
	13	ATA26D07	D13S779
66	12	ATA27A06	D12S1042
	18	GATA88A12	D18S976
	13	GGAA29H03	D13S1493
67	12	GGAT2G06	D12S398
	19	MFD235	D19S245
	13	GATA29A09	D13S788
68	18	ATA1H06	D18S844
69	12	GATA63D12	D12S1064
70	18	GATA64H04	D18S877
	12	GATA4H03	D12S372
	13	GATA23C03	D13S787
71	13	AFM309VA9	D13S285
	12	GATA13D05	D12S392
	18	ATA82B02	
72	X	GATA165B12	
	13	GATA7G10	D13S317
	12	GATA32F05	D12S2078
	18	GATA178F11	
73	20	AFM046XF6	D20S171
	22	GCT10C10	D22S1685
	Y	GATA30F10	DYS389
	X	GATA72E05	DXS7132
74	22	GGAT3C10	D22S445
	X	GGAT3F08	DXS9900
	21	GATA70B08	D21S1446
75	21	GGAA3C07	D21S1437
	22	GATA6F05	D22S685
	X	GATA182E04	DXS9908
76	21	GATA129D11	D21S2052
	18	GATA11A06	D18S542
	20	GATA42A03	D20S478
77	21	GATA188F04	D21S2055
	XY	SDF1	DXYS154
	20	GATA45B10	D20S480
78	22	AFM217XF4	D22S420
	3	AFM036YB8	D3S1259
	5	GATA2H09	D5S816
79	20	AFM077XD3	D20S103
	21	GATA11C12	D21S1432
	Y	GGAAT1B07	
80	X	GATA172D05	
	10	GATA87G01	D10S1432

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